NUCLEAR DIVISION IN ZYGNEMA.

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(WITH PLATES III AND IV)

THE species of Zygnema chosen for this investigation possesses a nucleus unobscured by chromatophores, and hence one in which division stages can be easily followed. No zygospores were found in the material, so the species could not be identified with any degree of accuracy. The number of pyrenoids are normally two, one on each side of the nucleus. The material was gathered from the same locality, the margin of a brook, during the months of August and September of two successive years. The filaments were studied in a living condition to make sure of the presence of dividing nuclei, and were then killed in chromacetic acid and the weaker solution of Flemming for later study. The greater part of them were killed in the evening, as it was also desired to secure division stages of other Conjugatae, which grew in great abundance in the locality and have been reported by investigators as dividing more actively at night. Of these, three species of Spirogyra and two of Mesocarpus will furnish the material for a later contribution.

As nearly all the literature upon the cytology of the Conjugatae relates to forms of Spirogyra, its consideration will be deferred until the completion of further studies in the nuclear division of the group. It is hoped then to bring into accord all the observations as to the character of chromatin and nucleoli.

Filaments of Zygnema treated with the combination stain of safranin and gentian violet, were found upon examination to have retained the violet only in the cell sheath, while the nuclear structures and pyrenoids retained the safranin. Various results were obtained with those treated with Heidenhain's haematoxylin in combination with iron alum and eosin. As the same length of exposure to the stain did not suffice for Spirogyra and Mesocarpus growing entangled with the Zygnema, the material was allowed to remain in the staining fluids for a shorter or longer time. Filaments show pyrenoids stained black by the haematoxylin, the nuclear structures retaining the eosin; or the pyrenoids may be stained red by the eosin, and the

nuclear structures black by the haematoxylin; or finally both may appear stained red by eosin. Such differences are shown in the drawings from the different preparations; the parts shaded in black represent portions stained by the haematoxylin, as in *fig. 33*, those in gray the portions stained by the eosin, as in *fig. 13*.

Within a quiescent nucleus situated between the two pyrenoids thus stained, there can be seen a central body stained somewhat redder or blacker, as the case may be, than the peripheral network of granules. This network of granules, ordinarily scarcely distinguishable from the cytoplasmic reticulum, was found in some cases to be quite conspicuous.

If an examination is made of a nucleus in process of reconstruction from the telophase, within the forming membrane can be seen a conglomerate mass of substance, very evidently non-homogeneous both in surface view and as seen in outline, *figs. 1, 39*. Around this smaller bodies can be seen in the meshes of a delicate network. The staining capacity of the larger mass and the small bodies varies in the different preparations; in some instances they are sharply defined from one another, at other times they retain the same kind and amount of stain.

It cannot be denied, however, after a careful examination of stages preceding the appearance of these bodies, that the substances in both came from the chromosomes of the metaphase. Bearing in mind, then, that the large mass and the smaller granules have the same origin, it would hardly seem correct to discriminate between the two, terming the one nucleolus and the others chromatin granules. Neither method of staining nor study of their history yields evidence other than that they are of similar substance, differing only in position and aggregation. It is as if in the revolutions going on within the cell some of the chromatin granules had been drawn to the center, there incompletely cohering, while others were left at the periphery. In describing, then, the quiescent nucleus of Zygnema it seems preferable to say that the larger portion of the chromatin granules cohere to form a central body analogous in its position to the nucleolus of higher plants.

The division of the nucleus is presaged by granules collecting in the region where the cell wall will form. The activity of these vibrat-

ing granules in the living cell renders the nuclei about to divide easily distinguishable from the remainder in the filaments. Owing to the activity of these granules, changes going on within the living nucleus could not be easily followed, but changes in the form and position of the nucleus together with those of the pyrenoids were followed throughout division. Accordingly the history of changes in the chromatin is all deduced from comparison of dividing nuclei stained by haematoxylin or safranin as outlined above.

If haematoxylin in combination with iron alum could be considered as an infallible criterion for distinguishing chromatic from achromatic material, and stages could be selected from material stained by one of the methods only, it would be an easy matter to trace the history of this central body originating from the chromosomes of the metaphase. Often, as in *fig. 2b*, numerous deeply stained bodies are to be seen lying in the space surrounded by a membrane, with no trace of chromatin bodies without. In the nucleus represented in *fig. 5*, in place of the central body several smaller bodies can be seen marked off from the eosin-stained bodies by the blackness of the stain. Passing to *fig. 9*, where the beginnings of an intranuclear spindle are manifest, and where there are several more deeply stained bodies, and then to *fig. 12*, where six discrete bodies distinctly form an equatorial plate, the natural conclusion, based wholly upon similar staining properties, would be that the central mass of chromatin alone furnishes the chromosomes for the equatorial plate. Such was the conclusion reached during the first year of this investigation, but further study of the material shows it to have been premature, or, if applicable at all, only to a few cases. The conviction that difference in staining of nuclear structures is more often a matter of manipulation than of chemical reaction, and that difference in the shade produced by the stain is merely due to the density of the body and time given for penetration, renders necessary in interpretation a great degree of caution.

The following account is derived from a comparison of parallel stages in all the preparations.

As the nuclei pass from the quiescent to the active state, the centrally lying mass disintegrates into small bodies (*figs. 2, 3*); at the same time the granules lying at the periphery increase in size. The

space within the nucleus becomes gradually clearer (*fig. 5*), the nuclear sap probably reinforcing the substance of the granules. As the result of the disintegration of the central body and the growth of the other granules, there may be seen lying within the nucleus twenty or more granules (*figs. 4, 5, 6*). In a few cases these bodies may slightly cohere, but in the majority of cases they lie free. No cases were found here or in later stages of the formation of a spirem. In many instances all the bodies within the nucleus retained merely the eosin stain (*fig. 6*), and hence were entirely undifferentiated from each other. In a few cases, like *figs. 5, 7, 8*, some of the bodies retained only the black stain from the hæmatoxylin. In one instance (*fig. 11*), a faintly stained larger body, with one or two smaller ones of similar shade, can be seen lying within the nuclear space, surrounded by numerous more deeply stained granules. If the other stages mentioned had not been observed, the latter faintly stained body might have been interpreted as a nucleolus like those in higher plants, now in the act of becoming dissolved in the cytoplasm. Extended comparison, however, of parallel stages justifies the view that this body is only a portion of the central mass of the quiescent nucleus, about to undergo still further disintegration into chromosomes.

The many chromosomes thus resulting approach one another (*figs. 6, 9*), presenting in many cases an appearance analogous to the synapsis stage described as occurring in the higher plants. Finally they become arranged in a circle concentric with the short axis of the cell. In one case (*fig. 10*), such an arrangement was observed before the nuclear membrane became dissolved. *Fig. 14* shows this massing of granules in the equatorial plane after the dissolution of the nuclear membrane. The chromosomes in this cell were all stained black, but some were drawn in lighter tint to show that they were lying in three different planes. *Fig. 15* also represents a similar stage, and *fig. 18* one somewhat further advanced. The chromosomes now appear to be denser than in previous stages, an interpretation based upon the circumstance that the hæmatoxylin stain does not as readily become washed out.

After having formed the ring they appear to be drawn inward, becoming denser and undergoing a process of fusion. By this draw-

ing-in process they come to lie in two closely adjoining parallel rows. As no case of a single row of isolated granules in the same plane was found, there is no evidence that such double row was produced by the division of a single row. *Fig. 25* represents two rows of chromosomes lying in the same plane. In *fig. 12* fusion has taken place to such an extent that only three chromosomes are present in each row.

Many of the chromosomes presented a tetrahedral appearance, as in *figs. 16* and *20*, thus pointing to the conclusion that the fusion of the condensing granules may take place in fours. In some cases the fusion has gone so far as to result in only four groups of tetrads (*figs. 22, 23, 21*). *Fig. 24*, of a more highly magnified group, shows especially distinctly this grouping of the granules. Careful focusing on this stage indicated the presence of another underlying group. As many cases were found of such grouping of the chromosomes in fours, it does not seem that it could have been purely accidental.

When the maximum amount of fusion and condensation is reached, the limit apparently varying in different cells, each half of a group becomes dissociated from its adjoining members and gradually draws away, as in *fig. 27*. In process of separation each group becomes broken up into smaller groups, in the meantime all becoming again arranged in two rings concentric with the short axis of the cell (*fig. 26*).

Thus numerous chromosomes are arranged in a circle in stages preceding and immediately following the stage of the equatorial plate, in which commonly four to six chromosome groups may be seen. It seems difficult to believe that six chromosomes (*fig. 12*) could have resulted directly from condensation and fusion of thirty or more chromosomes (*fig. 14*). A comparison of chromosomes as to size and staining qualities in the two adjoining cells (*figs. 12, 13*), drawn with Abbé camera, would certainly indicate that each chromosome must suffer a loss of its more liquid substance in the process of being drawn into the equatorial plate, or that a few must be entirely dissolved. Whether all condense to form a few, or whether only a few are chosen to transmit the chromatin to daughter nuclei, the remainder becoming dissolved in the cytoplasm, cannot be stated with certainty, as the staining process does not solve the problem as to the fate of the individual granules. When *all* the preparations are

examined and not a selected few, there seems to be more evidence of the first being the true account of events.

It was thought at first that this difference in number of chromosomes might be due to difference of species, as none of the Zygnema examined had zygospores, and hence it is possible that two or more species might have been growing together. The discovery of cells like those in *figs. 6, 12, 13, 15, 23, 22*, in the same filament is indisputable evidence that in the same individuals the number of chromosomes decreases from thirty or more down to six or eight, and then increases to thirty or more. This change in number occurs in a few moments, as determined in living cells by the changes in the position of the nucleus. All the filaments were examined in surface view, so it cannot be maintained that the number of chromosomes had been increased by sectioning.

As the rings of chromosomes approach the chromatophores, the cytoplasm is condensed on the side nearest the chromatophore. The explanation of this might be that a large part of the cytoplasm which is not diverted to the region of the formation of the cell plate was streaming in toward the center, as in *figs. 14–18*, while in *figs. 26–30* it was streaming out towards the chromatophores; that the chromosomes are forced together by the inflowing streams and in the vortex of opposing currents become dissociated. The word "dissociated" is used in preference to the word "splitting," as there appears to be no evidence of splitting and hence of equal distribution of homogeneous bodies. The chromosomes being heavier than the cytoplasm, the condensation appears on the side nearest the chromatophore (*figs. 28, 29*).

It is to be regretted that in the living cells chromosomes could not be distinguished from actively vibrating granules in the cytoplasm. Nothing could be discovered which in any way resembled spindle fibers, although streams of granules and the alternating space of nuclear activity was easily traced.

The number of chromosomes finally arriving at the chromatophore may be fifteen to twenty in each ring, as in *fig. 30*. The cytoplasm, being somewhat arrested in its flow by the chromatophore, causes a change in the position of the chromosomes. The majority, as they undergo still further dissociation, are drawn to the center, incom-

pletely cohering, while a few appear lying in delicate strands about them (*fig. 31*). In some cases all the chromosomes may cohere to form the central body. The nuclear membrane now emerges from the condensation of cytoplasm (*fig. 32*). As the chromosomes are now shut off from the influence of currents in the cytoplasm they generally remain unchanged in position, fusing either to form one mass (*fig. 33*), or three or more smaller masses (*fig. 34*), or rarely (*fig. 35*) all the chromatic material may be diffused in the nuclear plasm, forming numerous more or less tetrahedral granules.

It is to be noted that not until the chromatic rings have separated and have approached the chromatophores do the pyrenoids ordinarily show any evidence of division. This observation was easily confirmed from the study of living cells. *Fig. 29* represents the only one seen, out of many filaments examined, in which the pyrenoids divided before the formation of the nuclear membrane. As the newly divided nuclei approach their respective chromatophores, one or both plastids begins to show a constriction. This deepens until when the nuclei come to lie directly over, only a narrow band of less dense substance resembling linin connects the two daughter pyrenoids (*figs. 37, 38*). This becomes gradually reduced until it appears only as a thread (*fig. 39*). Later the nucleus sinks down and the separation is complete. The constriction of the plastids forming the center of the two pyrenoids takes place synchronously, as is the case with the stages in the daughter nuclei. One instance only was observed in which one plastid suffered division when other plastids had just begun to elongate (*fig. 32*).

Although division of the pyrenoid may be influenced by division of the nucleus, that it is not wholly dependent upon it was demonstrated by leaving actively dividing filaments of Zygnema for one hour in a watch crystal containing 10^{cc} of water to which two drops of chloroform were added. There were but few visible signs of plasmolysis in filaments killed and stained as in other material, but while a majority of the nuclei had ceased to divide, a majority of the pyrenoids were dividing as in normal filaments. That this division was not merely fragmentation was shown by sequence of stages and the presence of the band connecting the plastids. Fragmentation of the pyrenoids took place in filaments in stronger solutions of water

and chloroform in which plasmolysis occurred to a much greater extent.

Hence, cytoplasmic streams, nuclear structures, chromatophores, and pyrenoids take an active part in the division of cells in Zygnema. The streams of granules, collecting where the cell plate is to form, marks the beginning; the nuclear changes then proceed, followed by division in chromatophores and pyrenoids, while all are correlative with the formation of the cell plate.

It cannot then be said that division of the nucleus, the chromatophores, and the pyrenoids are synchronous. Rather is it true that the center of activities of the cell shifts, and with this shifting division of the bodies lying in the vicinity occurs. As regards the nuclear structures in Zygnema it is apparent that there are no bodies analogous to the nucleoli found in the higher plants. A large portion of the chromatin, or in a few cases possibly all, fuses in the anaphase to form one or more bodies corresponding in appearance and position to that of nucleoli of higher plants. Instead of waste products of chromatin condensing to form one or more bodies in the nucleus, the waste products are not separated from the chromosomes, but retained in them until after the nuclear membrane disappears in the next division. The substances which make up chromosomes and nuclear waste products, if such we may rightly regard the nucleoli of higher plants to be, are in Zygnema morphologically indistinguishable.

The history of chromatin before the formation of the equatorial plate may be summarized as consisting of growth, association, and condensation of chromatin bodies in groups. These groups may be partially coherent, but in no case form a spireme. After equatorial plate formation, dissociation into groups follows, continuing until the chromosomes reach the chromatophores.

Although the term chromosome has been used in this account, researches as yet incomplete make it exceedingly doubtful whether the chromatin bodies in any of the Conjugatae are to be regarded as at all homologous with chromosomes of higher plants. If we restrict the term chromosomes to segments of the tubular spirem,[1]

[1] See MERRIMAN, Vegetative cell division in Allium, BOT. GAZETTE **37**:178-207. *pls. 11-13.* March 1904.

then the chromatin bodies seen in *figs. 14* and *15* of Zygnema cells are homologous not with the chromosomes of Allium but with the granules seen in the earliest stage of the spirem, while groups in *figs. 16* to *23* are directly comparable with the groups or rings of tetrads, which in Allium fused to form the tubular chromosomes.

Zygnema possesses a mechanism of nuclear division less elaborated than that of the higher plants, inasmuch as dissociation of chromatin bodies occurs immediately after their association into primary groups without the intervention of a spirem. From this point of view appearances observed in Zygnema support the interpretation suggested in my account of nuclear division in Allium, namely, that the chromosomes are formed by fusion of bodies in groups, and that when a longitudinal splitting appears it is not to be considered a true splitting of a homogeneous substance but rather a dissociation of bodies which from the first were discrete.

If this be true, then doubts may reasonably be entertained as to the validity of the conception held by Roux, and successively by many other investigators, that the complex apparatus for indirect division of the nucleus exists for the purpose of enabling each chromatin body to furnish its quota to the daughter nuclei.

The essential feature of indirect division, and therein its advantage over direct division, appears to be the dissolution of the nuclear membrane. Thus is made possible a free interchange of nuclear and cytoplasmic substances and a renewal of the vitality of the cell.

Zygnema, then, may be considered as furnishing additional evidence of interchangeability of nucleoli and chromatin bodies, of variability in their number, and negatively as furnishing no evidence that equal distribution of chromatin is effected by either transverse or longitudinal splitting of homogeneous bodies. Nuclear structures, cytoplasm, pyrenoids, and chromatophores are transferred in equal amounts to the daughter nuclei and by a process differing not fundamentally in the result from that which would have been attained by direct division.

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EXPLANATION OF PLATES III AND IV.

The figures were drawn with the aid of an Abbé camera.

PLATE III.

FIG. 1. Daughter nucleus from a cell where the cell plate is not yet completed. The nuclear structures in this cell retained the eosin stain, the pyrenoids black from haematoxylin. × 1750.

FIG. *2a.* Nucleus preparing to divide, showing growth of bodies in the peripheral network before breaking up of the central body. Pyrenoids and nuclear structures in this cell retained only the eosin stain. × 1750.

FIG. *2b.* Nuclear material stained black by the haematoxylin, all the chromatic material being apparently condensed in the space occupied by the central body. × 1750.

FIG. 3. Nucleus showing the breaking up of chromatin body and increase in size of the peripheral bodies. The pyrenoids retained the eosin stain; all the nuclear structures are stained black, several of them somewhat darker than the others. × 1750.

FIG. 4. Nucleus showing the beginning of the massing of the chromosomes, the nuclear membrane as yet undissolved, the granules in the region of the cell plate formation being conspicuous. Chromosomes black, pyrenoids red.

FIG. 5. Later stage, showing the clearing of the nuclear interior, recalling the synapsis stage described in higher plants. Pyrenoids red, several chromosomes black, remainder red. × 1750.

FIG. 6. Similar stage, very frequent; chromosomes numerous, massed together, all stained red. × 1750.

FIGS. 7, 8. Similar stages where there is no massing of the chromosomes. In 7, six chromosomes were stained black, others red. In 8 those stained black are grouped in one corner of the nucleus, those red are scattered. × 1750.

FIG 9. A stage where distinct lines of granules connect chromosomes with nuclear membrane. Four chromosomes black, others red. × 1750.

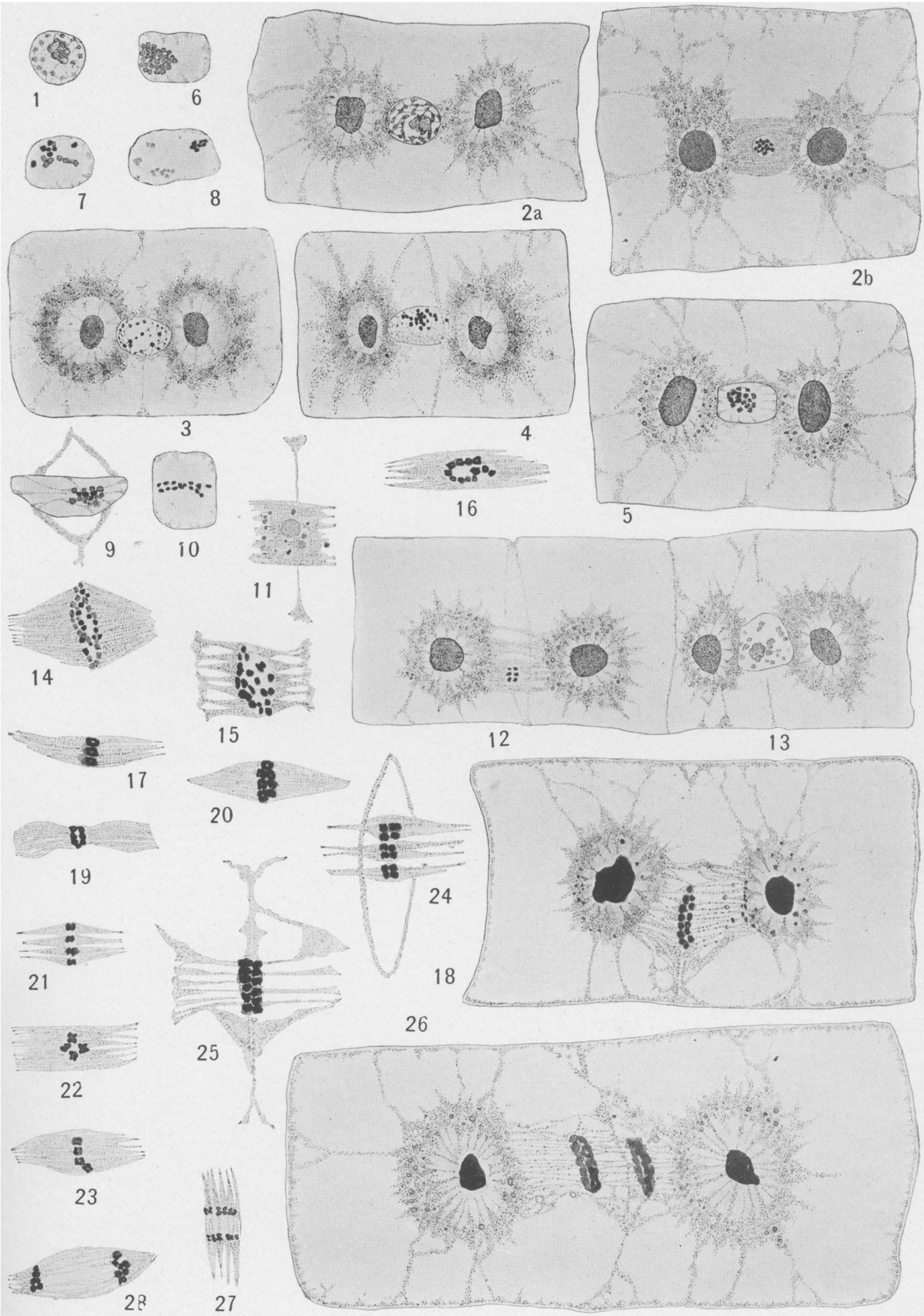
FIG. 10. A rare stage with numerous chromosomes arranged in circle within the nucleus before the nuclear membrane becomes dissolved. All chromosomes black. × 1750.

FIG. 11. Another rare stage; nuclear membrane dissolved, remains of central body still in the cytoplasm, retaining a lighter eosin stain than the other chromosomes. × 1750.

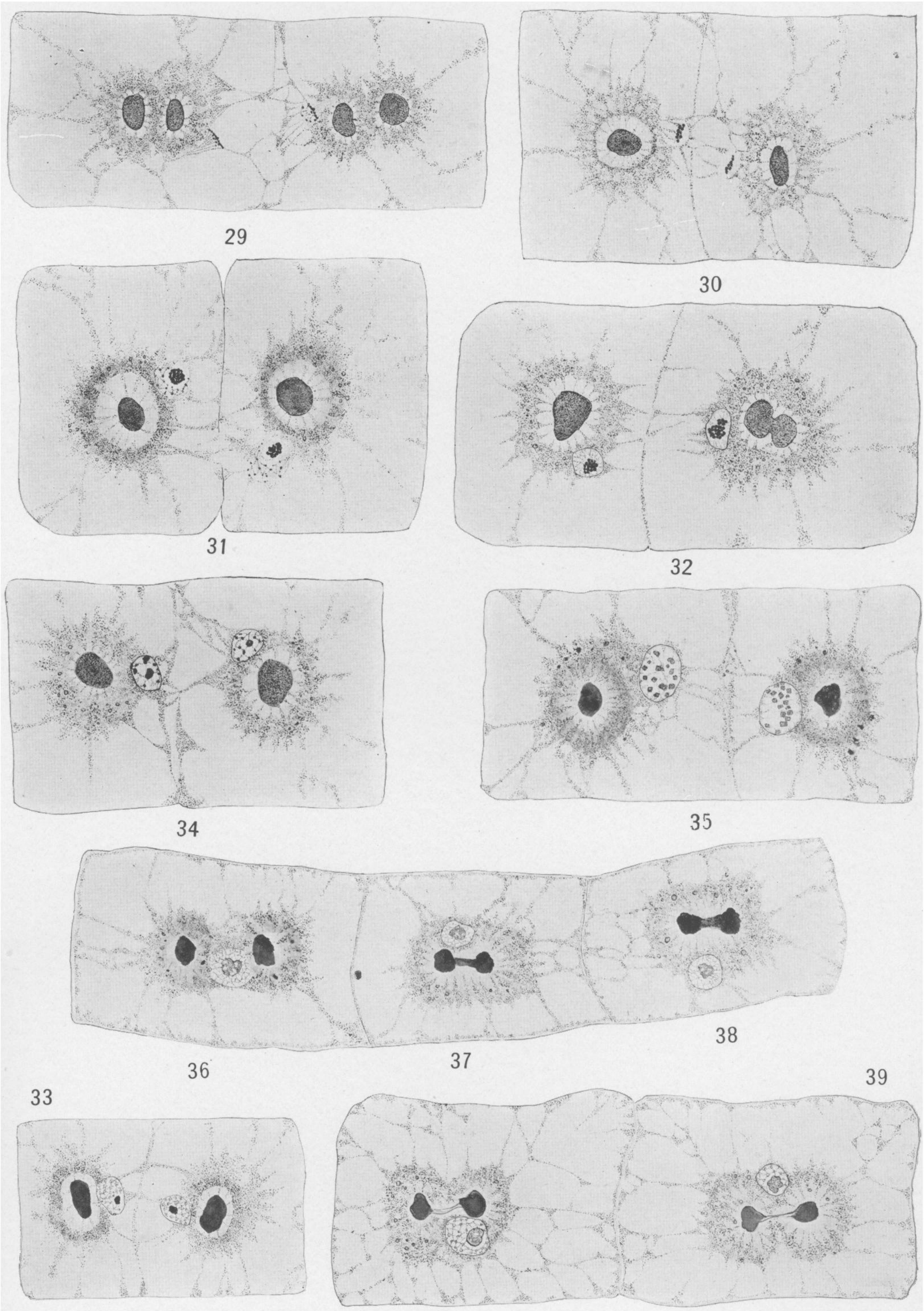
FIGS. 12, 13. Two adjoining cells in same filament showing disparity in size and number of chromosomes. Pyrenoids red, in *fig. 12* chromosomes stained sharply by haematoxylin; in *fig. 13* nuclear structures stained red. The line of granules marking the region of cell plate formation shown in both figures. × 1750.

FIG. 14. All chromosomes black, but some drawn lighter to indicate that they were lying in three different planes. × 2440.

FIG. 15. Chromosomes black, showing indefinite arrangement as they are being drawn to the center. × 2440.



MERRIMAN on ZYGNEMA



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FIGS. 16, 17, 19, 20, 21, 22, 23 show successive stages in condensation of chromosomes. Chromosomes all black. × 1750.

FIGS. 18, 24, 25, 26. Chromosomes and pyrenoids black. × 2440.

FIGS. 27, 28. Chromosomes becoming dissociated into smaller groups. Chromosomes black. × 1750.

PLATE IV.

FIG. 29. An unusual case of division of pyrenoids before formation of membranes of daughter nuclei. Chromosomes black, both in central mass and in the periphery, pyrenoids red. ×1750.

FIGS. 30, 31, 32. Chromosomes black, pyrenoids red. ×1750.

FIGS. 33. Pyrenoids and central body of nucleus black, peripheral bodies red. ×1750.

FIG. 34. Pyrenoids red, all the nuclear bodies black. ×1750.

FIG. 35. Nuclear bodies red, pyrenoids black. ×1750.

FIGS. 36, 37, 38. All nuclear bodies red, pyrenoids black. ×1750.

FIG. 39. Nuclear bodies red, pyrenoids black, showing vestiges of connecting substance. × 1750.